FREEZING IN INTERTIDAL ANIMALS[1]

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Low temperature is frequently the chief environmental stress which eliminates a species in a given location. Marine life is generally exposed to a minimum of − 1.7° C., the freezing point of sea water. In the intertidal zone a much lower extreme must be met. On a high latitude shore in winter one finds an abundance of invertebrates exposed twice daily to a period of − 20° C. or lower. At these temperatures ice formation in the animals might be expected. This has been found to be the case and is the subject of this paper.

The observations on freezing in surviving macroscopic animals are scattered and frequently indefinite. The literature is reviewed by Luyet and Gehenio (1940) and more recently by Scholander *et al.* (1953). In spite of the large number of references only a few experiments are found in which quantitative ice measurements were made on surviving animals. Sacharov (1930) showed that as much as a third of the body water could be changed to ice in some insect larvae. More remarkable is the better than 90% frozen body water that Scholander measured in chironomid larvae. It seems likely that this large fraction of ice is present in the qualitative experiments reviewed by Luyet and Gehenio. These include the survival at liquid air temperatures of nematodes, rotifers, and tardigrades. Such forms would be expected to have a high water content.

In the Woods Hole region in winter the shore supports large numbers of mussels, snails, oysters, and barnacles. Many of these are high up on the shore where they are exposed to the air for six hours or more at low tide. They are frequently in completely exposed locations, where it seems they could not escape any adverse effects of the cold. The sea spray freezes on them and they may be imbedded in ice for days or even weeks at a time.

The rate of cooling of an animal can be estimated from the known values of heat conductivity in tissue and shell. This was done assuming that the animal is subjected to a sudden drop of 20°. Such is the case when it emerges from the water on the receding tide on a cold day. With even the larger mussels the temperature at the center will be within a degree of the air temperature after an hour's exposure.

To check these estimates the temperature of the interior of several molluscs was measured without moving the animals. For this purpose a small hole was drilled through the shell with the animal still in its natural location. A thermistor probe one mm. in diameter was inserted through this hole and could easily be pushed deep into the tissues.

Several such measurements were made on a day when the air temperature was − 15° C. The animals had been exposed for at least two hours by the receding

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tide. Specimens of *Crassostrea virginicus*, *Mytilus edulis*, and *Modiolus modiolus* showed without exception interior temperatures within a few tenths of a degree of the air temperature. There seems no reason to doubt that the smaller snails and barnacles are in the same condition.

Ice could also be felt with the thermistor probe. These animals were alive and appeared normal when brought into the laboratory. From this it appeared reasonable that internal ice formation can take place without being fatal to the animal. It was next undertaken to determine the amount of ice formed at various temperatures.

Method

Water, on crystallizing to ice, gives off heat and also expands. These two properties have been separately employed in determining the amount of ice in biological material. The dilatometer as used by Gortner (1937) and others measures the expansion directly. The flotation method of Scholander *et al.* (1953) determines the specific gravity change which results from this expansion. Both of these techniques require that the animal be free of gas bubbles. This seemed impossible to determine in an animal living in a shell, so it was necessary to use a calorimetric method.

When a frozen animal is introduced into the calorimeter a certain number of calories of heat are absorbed to raise its temperature to some value above 0°. Part of this represents the heat of fusion of any ice which may have been present in the animal. The rest is the result of the heat capacity of the animal and its shell. The specific heats of these components are uncertain so it is impossible to calculate the latter value. However, in this work it is measured directly by finding the calories necessary to warm the animal through a temperature range in which there is no ice formation.

An ordinary wide-mouth Thermos bottle proved to be an efficient calorimeter vessel. A mercury thermometer through the stopper was read to .01° with a lens. By varying the amount of water in the calorimeter different sized animals could be studied efficiently. The freezer compartment of a home refrigerator was used to cool the animals. By adding additional insulation the temperature variation during the cycling of the refrigerator thermostat was less than 0.25° C. To minimize heat loss the animal was transferred to the calorimeter at the door of the refrigerator with cold tongs.

The calorimeter was calibrated by dropping weighed amounts of ice into it. Small cups were pressed from aluminum foil. These were filled with fresh water and frozen to a known temperature. The amount of heat absorbed in warming one from a temperature T_1 below 0° C. to a temperature T_2 above 0 is equal to:

$$C = M_w(T_1 \times .49 + T_2) + M_w \times 79.6 + M_{al} \times .21(T_1 + T_2)$$

where: C = gram calories
M_w = mass of water
M_{al} = mass of aluminum cup
.49 = average specific heat of ice
.21 = specific heat of aluminum.

The heat capacity of the calorimeter is then computed from

$$\text{heat capacity} = \frac{C}{T} \text{ calories/degree}$$

where T is the temperature drop in the calorimeter vessel resulting from the introduction of the ice.

If the heat capacity of a specimen were constant with temperature a number of calories equal to $(T_2 + T_1)$ times its heat capacity would be required to warm it. All calories in excess of this could then be attributed to the change of state from

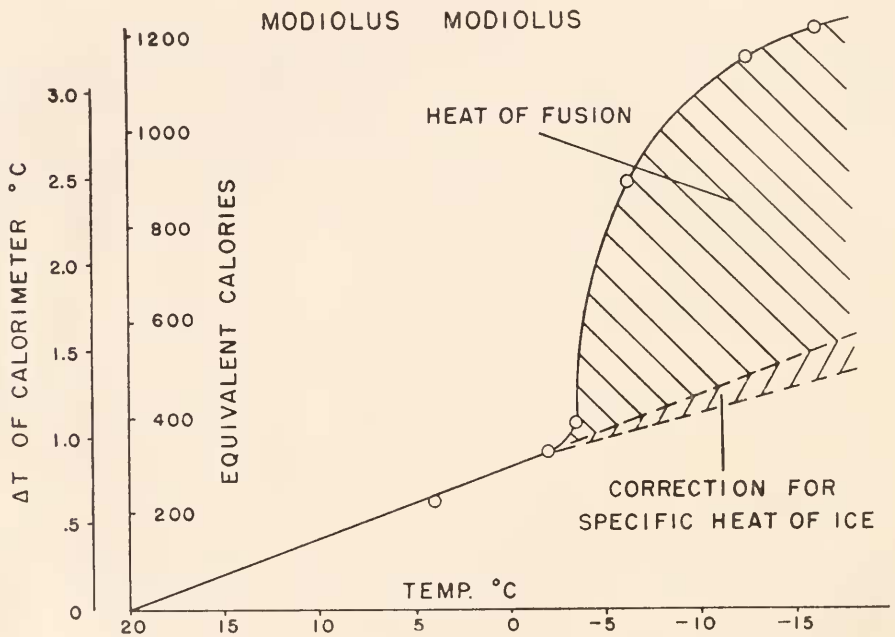

FIGURE 1. Graph showing ice formation in the mussel Modiolus modiolus. The shaded portion represents the calories used in the melting of ice. All points are from a single individual. The heat capacity of the animal changes as more ice is formed, resulting in the correction shown.

ice to water. This is not strictly true since the specific heat of ice is half that of water. As more ice is formed the heat capacity of the animal is correspondingly lowered. This results in the correction shown in Figure 1. The rigorous equation of Ditman *et al.* (1942) is difficult to apply and in actual practice some of the terms are very small. The graphical method used here is believed to have an accuracy commensurate with the experimental technique.

Ice determinations were made on animals frozen normally on the shore. They were placed directly in the calorimeter with a heavily gloved hand. Values obtained were essentially the same as for the animals frozen in the laboratory.

The bivalves were wedged open and all excess sea water forcibly shaken from them. They closed normally when the wedge was removed. They were then

wrapped with a layer of self-vulcanizing rubber tape which provided a water-tight cover with a minimum of insulation. This cover allowed the animal to be removed from the calorimeter and refrozen at a different temperature without varying the amount of water in the shell.

In order to work with a single individual of the smaller species, such as Littorina, a method of micro calorimetry was worked out. The calorimeter vessel consisted of a round bulb on the end of a long thin-walled glass tube. Loose cotton insulation was packed around this. Such an arrangement was found to have a desirably small temperature drift. In a small calorimeter vessel the ratio of surface to volume becomes unfavorably large. It can be shown that most of the heat leakage is by way of conduction through the wall forming the opening to the chamber.

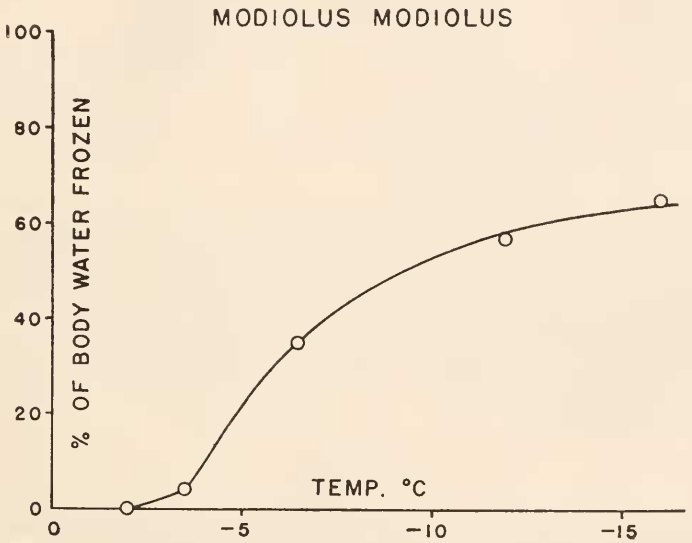

FIGURE 2. Graph showing the percentage of water as ice. The calories of the shaded portion of Figure 1 were converted to grams of ice and expressed as the percentage of the total water in the animal.

To reduce this the thinnest glass tube possible was used. The methods employed on larger systems, such as silvering and operating in a vacuum, do not appreciably improve a small calorimeter. The volume of the bulb varied from 10 to 25 cc. depending on the size of the specimen. The temperature was measured with a thermistor suspended in the water. The same method of calibrating with a weighted amount of ice was used.

RESULTS

Figure 1 is typical of the results obtained. It is a graph of the amount of heat necessary to warm the animal up to 20° C. after it has been equilibrated for several hours at some lower temperature. Since the specific heats of tissue, shell, etc. are not appreciably altered by temperature one might expect a linear relation-

ship. The amount of heat should depend only on the temperature range if the heat capacity of the system is unaltered. However, below 0° a larger number of calories than expected is absorbed. It is this additional heat that is required to melt the ice in the animal. Its amount can be found from the curve at different temperatures and converted to grams of ice. Since the total body water is found at the end of the run it is known what fraction of this is frozen at the various temperatures. Figure 2 is the result of such a calculation from the data in Figure 1. All points on a curve such as the one in Figure 1 are measured on a single animal. The data were not considered if the animal was not alive and of normal appearance at the end of the run.

Table I

Percentage of water frozen at —15° C.

Species	% frozen at −15°
Mytilus edulis	62
Modiolus modiolus	65
Littorina littorea	59
Crassostrea virginicus	54
Littorina rudis	67

Table I gives the fractions of body water as ice at − 15° C. in the different species examined. Individual measurements were made on Modiolus and *L. littorea* at − 22° C., the lower limit of the refrigeration equipment. The fractions of body water frozen at this temperature were 71% and 76%, respectively. The animals survived several days exposure to this temperature. *Littorina rudis* was studied at Hebron in Labrador while on an expedition sponsored by the Arctic Institute of North America.

Discussion

Bachmetjew (1899), Salt (1950), and others have claimed super cooling of animals as great as − 15 to − 20° C. However, they expressed the strong belief that freezing was fatal and seemed to reason inversely that when the animal was dead it was therefore frozen. Ditman *et al.*'s (1942) calorimetric work represents one of the few quantitative ice studies. It was necessary for them to use a number of insects simultaneously in the calorimeter. They reasoned that the appearance of more and more ice at lower temperatures was due to variability in the super cooling of the individuals. Their curves indicate 70% frozen body water for coddling moth larvae at − 18°. This seems at variance with their conclusions of super cooling. This is not supplemented with measured lethal low temperatures although in a later paper Ditman shows complete survival of this species at this temperature.

The values in Table I show large amounts of ice at − 15° in all the forms that could stand freezing. The shape of the ice-temperature curve for the other species was the same as that in Figure 2. Some of the forms could stand a period at − 5° without ice formation. In no case, though, was this observed at − 7° or lower. Values of ice in the region of super cooling were obtained by cooling the animal to a lower temperature to start ice formation and then warming the

animal. Super cooling of shore animals in nature only appears to occur down to -7° as a maximum.

As much as 75% of the water in the animals can be tied up as ice. It is interesting to speculate on the severity of the physiological stress represented by this sudden dehydration.

With the living processes in the protoplasm deprived of this water one might expect a slowing down of the metabolic rate. Lichens have been shown to respire at a rate related to their moisture content (Smyth, 1934; Neubauer, 1938). Scholander *et al.* (1953) have shown a precipitous drop in oxygen consumption concurrent with ice formation in a chironomid larva. This resulted in an apparent Q_{10} as high as 50 in this low temperature range.

In an animal that is 75% frozen the remaining brine concentration is 4 times the normal value. Actually some of the salts will probably have begun to precipitate out at this temperature and concentration. One might suspect many dire consequences at such a high salinity. Lovelock (1953) has published results which indicate that the high electrolyte concentration is the destructive factor in the freezing of red blood cells. The dehydration and high salinity appear to be factors the intertidal species can tolerate, at least at these low temperatures.

Ice is thought not to occur inside cells without damage (Chambers and Hale, 1932; Asahina *et al.*, 1954). It seems reasonable that such a finely organized structure as protoplasm could not stand the physical disruptions of intracellular ice. Apparently the cells are able to allow for a rapid exit of water and to stand the distortion of intercellular ice crystals. Meryman (1953) has shown this situation in slowly frozen rabbit liver. In the thawed tissue the water has migrated back into the cells and the appearance is once again normal. Siminovitch and Briggs (1952) have related frost hardiness in plants with an increased ability of the water to diffuse in and out of the cells. Asahina and collaborators have observed both extra- and intracellular freezing in insects. The latter was always fatal to the cells involved. Proof of such a situation in the frozen shore life can only come from histological examination.

The ice determinations here are reproducible within a range of $\pm$ 5%. Weighed amounts of ice could be measured with a 2% error. However, the large amounts of shell and wrapping hinder thermal equilibrium and require many calories for their warming. This forms a background above which ice determinations in such animals must be made.

In Figure 3 the freezing curve for a mussel is reproduced with one for sea water. This latter was obtained by treating a capsule of sea water in the same manner as the animals, *i.e.*, it was cooled to different temperatures and warmed in the calorimeter. No freezing curve for sea water could be found in the literature. The mussel contains significantly less ice than sea water at all temperatures. Part of this may be due to the lack of thermal equilibrium in the animal, particularly on the steep part of the curve. However, the many theories of bound water lend support to the idea that the disparity of these curves is real. To some extent the body fluids can be considered salt solutions and to this extent such a freezing curve would be expected. Any departure may well represent the participation of water in living processes other than as that of a simple solvent. Such a molecule as water with its high dipole moment can be held in low energy fashions unlike conventional bonding. This would compete with freezing which is itself a low

energy per molecule process. When the physics of water in living systems is more thoroughly understood a closer approximation to the actual freezing curve can be theorized.

Attempts were made to freeze a large variety of molluscs, echinoderms, annelids, and arthropods which occur only below tide level. No animal was found to stand low temperatures and large internal ice formation that is not faced with these conditions in nature. The hardiness towards ice formation may be the principal reason allowing intertidal species to successfully invade the shore. Resistance to freezing runs parallel with the ability to withstand dehydration (Scarth, 1944; Siminovitch and Briggs, 1952; etc.). Both of these conditions are found in a situation of tidal exposure. The shore snails can be kept out of water for a period of weeks as can the mussels. However, all the forms frozen so far are encased in

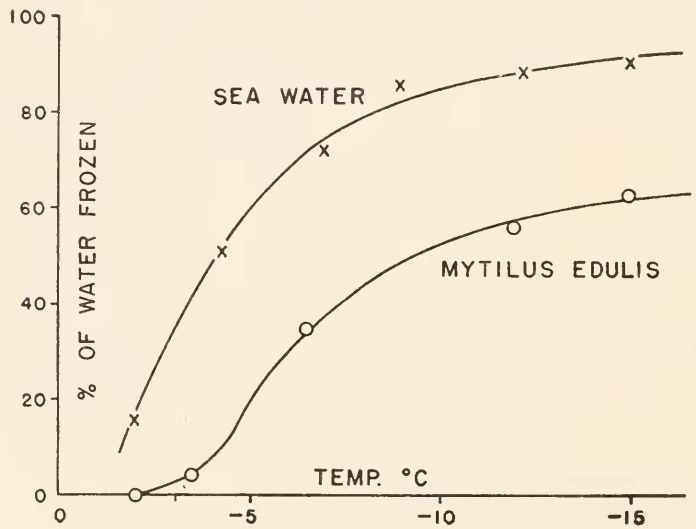

FIGURE 3. Graph of ice determined in the same fashion for a vial of sea water and a mussel. The difference is believed to represent unfreezable or bound water.

a shell and it is not certain how much natural dehydration they undergo independent of freezing.

Finally, some observations were made in the Arctic that are interesting. In this region of permafrost the only protection from freezing afforded poikilotherms in the winter is in the sea. Hebron Fiord in Labrador has a year round bottom temperature of $-1.7°$ C. In spite of this the extensive bottom invertebrate fauna could not stand freezing. Here are animals that live and breed right up against the freezing barrier, and yet the particular adaptations of the shore life are absent. The only two plentiful intertidal forms were Mytilus and Littorina. Both of these displayed their usual resistance to freezing. Beds of Mytilus were noted which spend 6 to 8 months frozen solid in the ground ice at temperatures of $-20°$ C. and below! In the spring the Eskimos chip them out for food.

I am greatly indebted to Dr. Per Scholander for his continued interest and help-

ful advice during this work. My wife has also been of great assistance, particularly under the trying conditions of cold winter days on the shore.

Summary

1. The temperature environment of shore life in the Woods Hole vicinity has been found to range as low as − 20° C. in the winter. *In situ* measurements have shown that no protection in the way of a micro climate is afforded most of this life.

2. A variety of intertidal forms has been frozen in nature and in the laboratory and their ice content measured. In live animals as much as 75% of the body water has been shown to be in the form of ice at temperatures regularly met in nature.

3. Some physiological consequences of this ice formation are considered.

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